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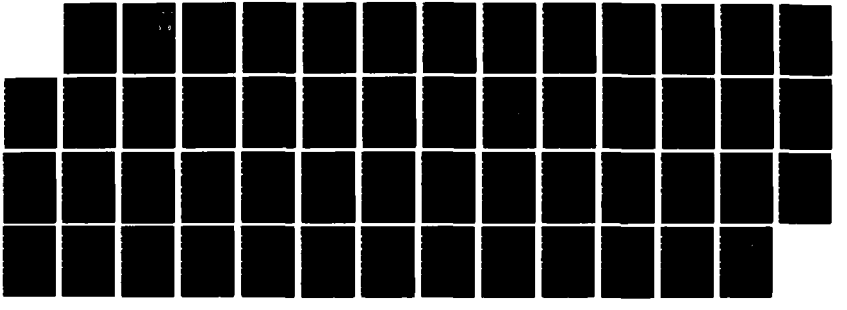

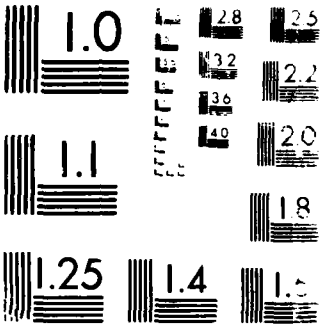
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TECHNICAL DEVELOPMENT AND APPLICATIONS OF NEW ADVANCES TO PROBLEMS OF FLUID/ELECTROLYTE/MINERAL IMBALANCE IN MILITARY PERSONNEL

AD-A186 437

FINAL REPORT

MORTEZA JANGHORBANI, Ph.D.

APRIL 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Grant No. DAMD17-85-G-5036

BOSTON UNIVERSITY SCHOOL OF MEDICINE
85 E. Newton Street, M1008
Boston, MA 02118

SECURITY CLASSIFICATION OF THIS PAGE

AD-A186437

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Boston University School of Medicine; 6b. OFFICE SYMBOL (If applicable):	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): 85 E. Newton Street, M1008 Boston, MA 02118	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: USAMRDC; 8b. OFFICE SYMBOL (If applicable):	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-85-G-5036
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 62777A; PROJECT NO. 3E162 777A879; TASK NO. BA; WORK UNIT ACCESSION NO. 098

11. TITLE (Include Security Classification)
Development and Applications of New ~~Technological~~ Technical Advances to Problems of Fluid/Electrolyte/Mineral Imbalance in Military Personnel

12. PERSONAL AUTHOR(S)
Morteza Janghorbani, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final Report	FROM 7/1/85 TO 1/1/87	April 1987	51

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Heat Research, Stable Isotopes, Electrolytes, Minerals, Trace Elements, Body Water
06	06		
06	19		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This constitutes the final report for the period 7/1/85 through 1/1/87 in the studies related to methods development for stable isotope tracers in heat research.

Four segments have been described in this final report: 1) acquisition, installation, and testing of a two-analyzer system for measurement of stable isotopes 2) development work related to stable isotopes of rubidium 3) development of measurement aspects of stable isotopes of bromine and 4) completion of analytical chemistry for stable isotopes of lithium.

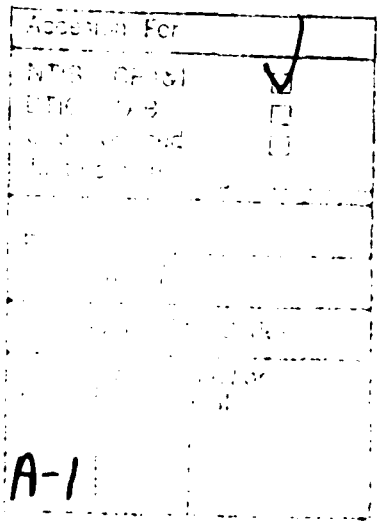
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Judy Pawlus	301/663-7325	SGRD-RMI-S

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

For the protection of human subjects the investigators have adhered to policies of applicable Federal law 45CFR46.



SUMMARY

This is the final report for the period 7-1-85 through 1-1-87 for grant DAMD17-85-G-5036. This report consists of four segments.

Segment - I-A two analyzer system has been acquired, installed, and tested for the measurement of stable isotopes.

Segment - II-Work is reported on the potential applications of stable isotopes of rubidium in studies of body potassium.

Segment - III-Methods are described for accurate measurement of stable isotopes of bromine for future studies of bromine space, extracellular fluid volume, and exchangeable chloride in man.

and Segment - IV-Methods are described for accurate measurement of stable isotopes of lithium for studies related to potentiating effect of lithium administration in induction of heat illness.

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Introduction

This constitutes the final report for research conducted under the auspices of Grant No. DAMD17-85-G-5036, for the project period 7-1-85 through 1-1-87. This should be considered as continuation of a previous final report, submitted for Grant No. DAMD17-84-G-5012, covering the period 6-18-84 through 6-17-85.

The overall objectives of the work conducted under the auspices of these grants have been to develop novel methods based on the concepts of stable isotope tracers for specific applications to studies of exercise under heat stress in military personnel. In the previous grant period (6-18-84 through 6-17-85) stable isotope methods were developed to permit initiation of studies related to dynamics of water transport in human subjects, using the $H_2{}^{18}O$-method. In addition, work was initiated to develop suitable measurement methods for other selected stable isotopes relevant to the broad objectives of this grant.

Following the successful outcome of $H_2{}^{18}O$-method development leading to subsequent application of the method to human studies (Contract No. DAMD17-86-G-6167), research was initiated to acquire a multi-faceted stable isotope ratio mass spectrometer with capabilities both for gas analysis (for work like $H_2{}^{18}O$) and thermal ionization (for studies of electrolytes, minerals, and trace elements). Simultaneously, work was continued to complete certain aspects of stable isotope methods development initiated under the previous grant (DAMD17-84-G-5012), and to initiate new methods development consistent with the long-term goals of the overall project.

The work reported in this final report consists of that portion of the overall investigations that have been successfully completed during the 18-month period of this grant. Additional work has been initiated, with the aim of completion during the follow-up phase of this project.

This report includes the following segments:

1. Acquisition, installation, and successful testing of the instrument for measurements of $^{18}O/^{16}O$.
2. Completion of methodology of $^{87}Rb/^{85}Rb$ measurements using both inductively Coupled Plasma Mass Spectrometry (ICP/MS) and Thermal Ionization Mass Spectrometry (TI/MS).
3. Successful completion of methods development phase of stable isotopes of Br ($^{81}/^{79}Br$) for later use with studies of Total Body Water; using the method of ICP/MS.

and

4. Initiation and successful completion of the methods phase of stable isotopes of lithium ($^{6}Li/^{7}Li$) for later use as possible model for inducing thermal stress in animals; instrumentation: ICP/MS.

The methodology for the work initiated under this program was not in existence prior to its initiation here. The concepts and developments are novel. Their applications to the field of heat research remain yet to be explored by any group. Applications of these novel methods are certain to permit conduct of research not possible prior these developments.

Segment I-Instrument Acquisition, Installation, and Testing

A two-analyzer system capable of isotope ratio measurements on both gases (for $H_2{}^{18}O$) and solids (for electrolytes, minerals, and trace elements) was acquired, installed, and tested for the immediate application to $H_2{}^{18}O$ measurements.

Typical performance data for the measurement of $^{18}O/^{16}O$ have been summarized in Table I. These data indicate that the instrument performance is quantitatively similar to other state-of-the-art instruments for this purpose.

This instrument is essentially two analyzers with a common set of inlet and electronics, capable of independent operation, following appropriate upgrading. Two modes of ionization are available: electron bombardment for gas analysis and thermal ionization. Present plans call for eventual upgrade of the instrument as two separate devices so that work in areas of $H_2{}^{18}O$ and minerals can proceed independently and without impediment.

Table I - Measurement Performance of $^{18}O/^{16}O$ Measurements

Sample ID	Measured Delta, per mil[1]	SD[2]
1, unspiked	15.463	0.053
2,	15.394	0.055
3,	15.507	0.086
4, spiked 1	58.270	0.041
5,	58.752	0.029
6,	57.733	0.027
7, spiked 2	173.826	0.038
8,	173.977	0.069
9,	174.272	0.078

[1]each data point corresponds to mean of three sets of measurement on each gas sample. [2]standard deviation of the three measurements in units of per mil.

Segment II - Studies with Stable Isotopes of Rubidium

Because of present lack of suitable measurement methods for stable isotopes of potassium for application to studies of exchangeable potassium (K_e), we have developed the concept of rubidium (Rb) as a potential tracer for K. In the present grant, we have developed the analytical chemistry of stable isotopes of Rb (^{85}Rb, ^{87}Rb), using both ICP/MS and TI/MS. Here we have given a report of the salient features of this new method.

The next phase of this work involves testing the hypothesis of equivalency of Rb and K as physiological markers, a task planned for the continuation phase of the program.

ANALYTICAL CHEMISTRY

Instrumentation. An Elan^{Tm} Model 250 ICP/MS System (SCIEX, Thornhill, Ontario, Canada) was used in the isotope ratio mode for isotopic analyses of $^{87}Rb/^{85}Rb$. Operating conditions of the instrument have been described previously, fully in segments III and IV of this final report. In general, ion intensities for each isotope peak were averages of three measurements each for three seconds (total count time for each peak was nine seconds). The instrument automatically corrects for any ^{87}Sr contributions to the ^{87}Rb peak by also monitoring ^{88}Sr peak intensity. Total Rb and K were measured by atomic emission spectrophotometry (AES) using a Perkin-Elmer Model 5000 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT).

Sample Preparation. Red blood cells and urine were prepared for isotopic analysis by ion exchange chromatography, as per scheme given in Fig. 1. The columns were regenerated between runs with 30 ml of 4N HCl, then rinsed with deionized water until the pH of the eluate was ~ 6. Recovery of Rb from the columns was in the range 65 - 100%.

In preparation for Rb analysis by AES, red cells were wet ashed with 5 ml of concentrated HNO_3 and 2 ml of 30% H_2O_2 and diluted with 2 x 10^4 ppm Na as NaCl. Urine was diluted with 1 x 10^4 ppm Na for Rb analysis. For analyses of K, nonashed red cells and urine were diluted with 100 ppm Li as $LiNO_3$. All samples were compared to standards of known Rb and K concentration as atomic spectroscopy standards (EM Science, Cherry Hill, NJ).

Chemicals. All chemicals were analytical reagent grade and used as purchased. $^{87}RbCl$ was purchased from Oak Ridge National Laboratory, Oak Ridge, TN, as a powder, with isotopic composition (weight %) of 98.04% ^{87}Rb, and 1.96% ^{85}Rb. Doses and standards of ^{87}Rb were prepared with deionized water.

Results and Discussion. Two methods appear to be potentially applicable to quantitative measurement of isotope ratio $^{87}Rb/^{85}Rb$: ICP/MS and TI/MS. We have pursued both these techniques and here provide comparative data on their performance.

For the measurements with TI/MS, high potassium content interferes with Rb measurements. A workable K/Rb ratio is about 10/1. In comparison, natural ratios of K/Rb in urine and red cells are about 1000/1. For ICP/MS, while it is possible to aspirate unprocessed body fluids directly into the argon plasma, the high-salt content may cause instrument instability. Thus, a modest separation of matrix constituents is deemed necessary. These considerations have led us to development of a suitable separation scheme which yields samples for introduction into both ICP/MS and TI/MS (Fig. 1).

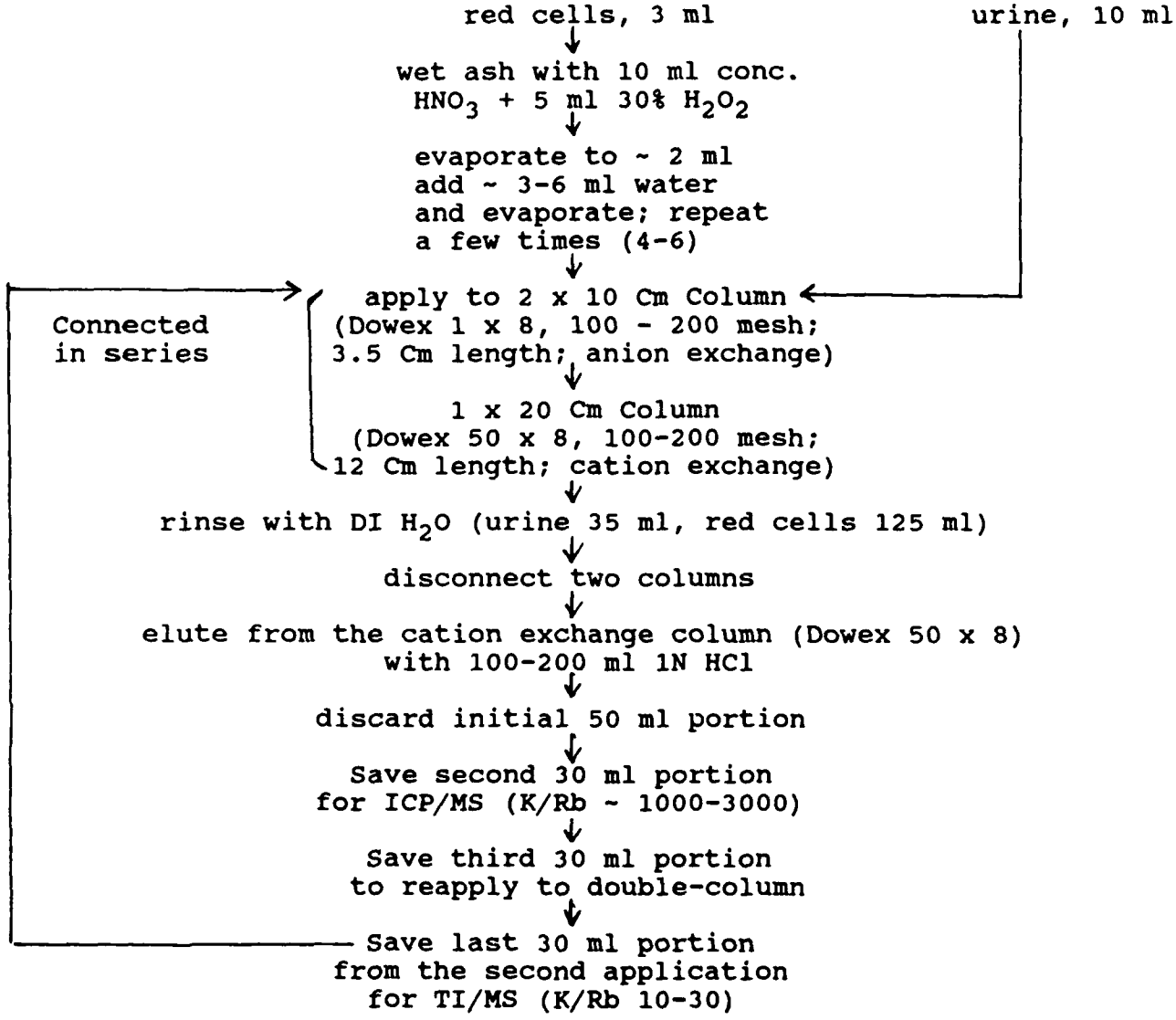

Fig. 1 - Scheme for Isotopic Analysis of Rb

Measurements with TI/MS. Data on reproducibility of measurements of the ratio $^{87}Rb/^{85}Rb$ are given for TI/MS in Table II for a number of materials. As indicated in the table, the reported values for each sample type represent the mean $\pm$ 1SD for the number of replications (actual sample loadings using an automatic multiple sample turrets system) indicated. These data indicate that under the conditions employed, the coefficient of variation for these measurements was in the range 0.2-0.5%.

Measurements with ICP/MS. TI/MS is a well established method of isotopic analysis in geochemistry. In contrast, ICP/MS is a new technique and its ability to accurately measure $^{87}Rb/^{85}Rb$ in biological samples has not been previously evaluated. As this is the first report of these measurements in relation to the present application, we have provided the necessary data to permit realistic evaluation of its performance characteristics.

Mass spectra generated from the argon-plasma possess special spectral characteristics which could become limiting in stable isotope tracer studies. Of the three types of potential mass spectral interferences involved with ICP/MS; viz. argon-plasma mass spectra, reagent generated mass spectra, and isobaric mass spectra, the former two do not appear to present any significant problem for the measurements of ^{85}Rb and ^{87}Rb. The only known potential isobaric interference is that from ^{87}Sr (nat. ab. 7.0 at .%). However, as clearly observed from the expanded scale of Fig. 2, which represents the mass spectrum from 10 ml fresh human urine processed according to the scheme of Fig. 1, the strontium contribution to ^{87}Rb is negligible. The ion intensity observed for ^{87}Rb in this run was about 30,000 ions/sec. As clearly seen, even for the major-abundant strontium isotope (^{88}Sr, nat. ab. 82.6 at .%) the ion intensity is negligibly small compared to that observed for ^{87}Rb. Similar results have been observed for red cells.

The mass spectrum for typical procedural blank is shown in Fig. 3. Similar blank spectra have been obtained on a number of occasions. Small mass ion peaks appear at mass numbers corresponding to ^{85}Rb, ^{87}Rb, and ^{88}Sr. However, the magnitude of these peaks is clearly negligible compared with the expected ion intensities for ^{85}Rb and ^{87}Rb.

Precision of isotope ratio measurements for $^{87}Rb/^{85}Rb$ using the ICP/MS was tested by replicate analysis of natural and ^{87}Rb-spiked samples (Table III). Each sample was processed according to the scheme given in Fig. 1. The intrasample precision (1SD of ten sequential ratio measurements on a single solution) of these measurements was 1%. The data show clearly that intersample coefficient of variation was in the range 0.3-0.8% (cf. data of Table II for TI/MS).

Table II - Precision of Isotope Ratio Measurements for $^{87}Rb/^{85}Rb$ with TI/MS[1]

Matrix	#Sample Loadings (n)	$^{87}Rb/^{85}Rb$ (mean + 1SD)
Standard Solution	6	.3895
of RbCl		.0020
RbCl (Fisher Scientific)	9	.3903
		.0009
Rb_2SO_4 (K & K Labs)	8	.3876
		.0013

[1]Measurements carried out by Dr. Harold Krueger (Krueger Enterprises, Cambridge, MA)

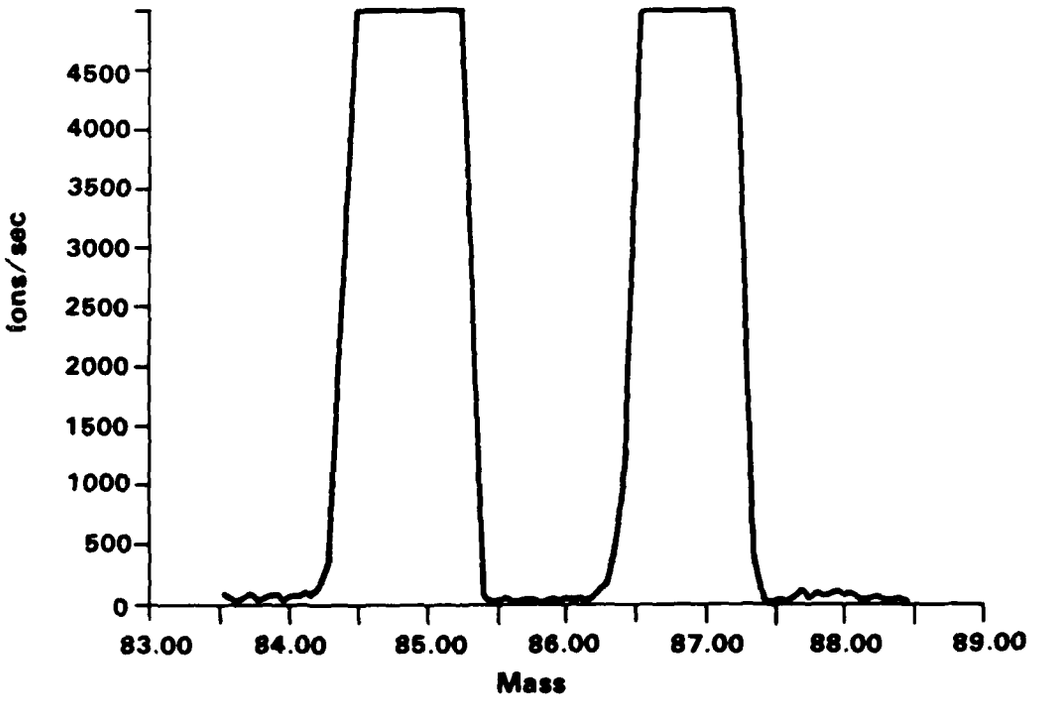

Fig. 2 - Human Urine Processed for Stable Isotopes of Rb

Employing graded ^{87}Rb-spiked samples of synthetic solutions, urine, or red cells, calibration plots expressing the relationship between the expected isotope ratio ($MIR_{87/85}$) and the measured ratio ($MR_{87/85}$) were constructed. These plots are given in Fig. 4. It is clear that highly linear calibration plots can be constructed. However, it is not clear from the present data whether these plots differ in detail for the various matrices of interest and whether matrix matching is actually required. This is because the calculated values of $MIR_{87/85}$ are uncertain to the extent that our analysis of red cell or urine rubidium may be in error (see section below). The maximum error resulting from any potential matrix effect can be readily estimated by considering the calculated values of $MIR_{87/85}$ at the upper end of enrichment, e.g. $MR_{87/85}$ of 0.80, between plots (a) and (c) For such a case, using plot (a) at $MR_{87/85}$ of 0.80 we would estimate the value of $MIR_{87/85}$ at .7447 while for plot (c) the corresponding value would be .7920, a 6.2% difference. In practice, calibration plots are made from the same matrix as the samples so that this issue does not introduce a source of concern.

Comparative Performance of TI/MS and ICP/MS. Employing the scheme of Fig. 1, ^{87}Rb-spiked urine and red cells were analyzed for their isotope ratio with both ICP/MS and TI/MS. The results have been plotted in Fig. 5 and indicate excellent agreement between the two methods. Thus, it is clear that TI/MS, as applied in this study, may possess somewhat better precision (Table II; observed range of coeff. of variation 0.2 - 0.5%) than that corresponding to ICP/MS (Table III; observed range of coeff. of variation 0.3 - 0.8%). However, under the conditions of this study, the observed precision performance of TI/MS is not dramatically superior to that for ICP/MS. The outcome of the somewhat improved precision in regard to its effect on the uncertainties of the estimates of rubidium space is practically negligible. The measured values of $MR_{87/85}$ in urine and red cells processed by a common scheme (Fig. 1) are practically identical between the two methods (Fig. 5). Thus, in terms of instrument performance, either method is as suitable for these measurements.

As clearly evident from the scheme of Fig. 1, application of TI/MS requires considerably greater separation of Rb from K. In contrast, ICP/MS does not possess a stringent separation requirement. In the present application, we have chosen a limited chemical separation as we are concerned about potential effects of high-salt solutions on the long-term performance of the instrument.

Accuracy of Rb Analyses. We have tested the issue of accuracy by analyzing samples of human urine for absolute Rb content by three methods: Isotope Dilution Analysis (IDA) employing ^{87}Rb as *in vitro* spike and ICP/MS; 2) Standard Addition using atomic emission spectrophotometry; and 3) Standard Curve employing atomic emission spectrophotometry. In the IDA method, the individual values of MR were converted to MIR using spiked

urine standard samples. The results of these analyses are given in Table IV. All methods resulted in similar values for urine Rb concentration. Therefore, the standard curve method of atomic emission spectrophotometry, the fastest and simplest technique, was used for subsequent determinations of total Rb.

Conclusions. The results from this work clearly demonstrate the utility of ICP/MS for accurate measurement of the two stable isotopes of Rb. The next phase of this project deals with establishment of the equivalency of the Rb-tracer approach with that of a tracer of potassium. This is being pursued at present.

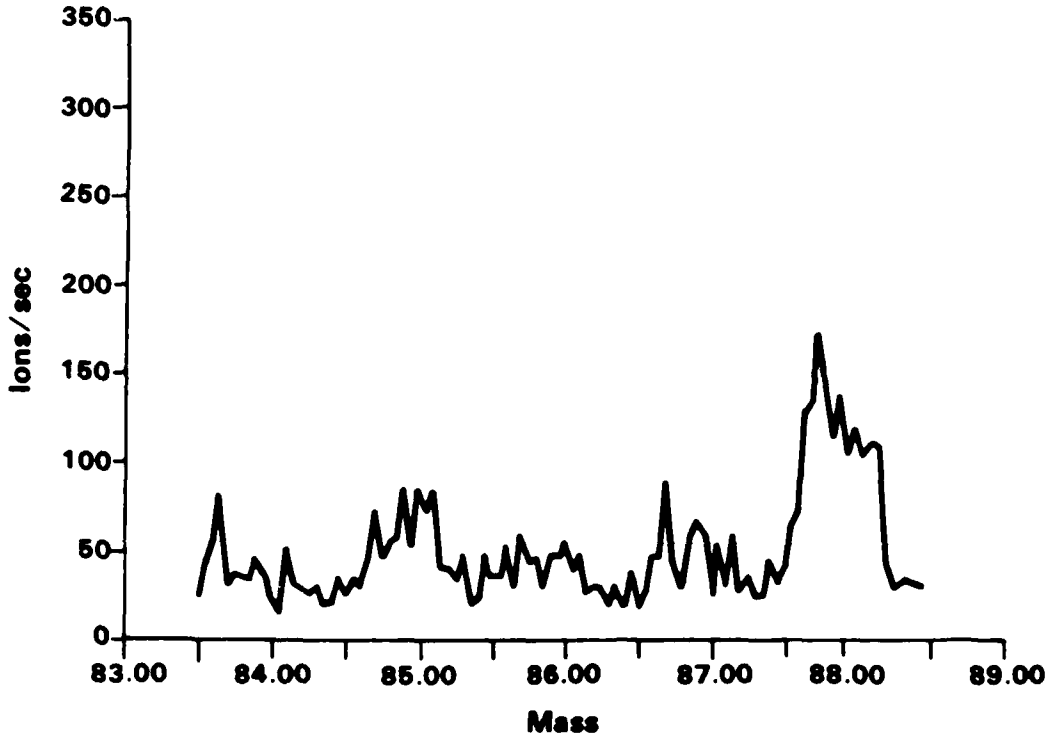

Fig. 3 - Mass Spectrum of Procedural Blank

Table III - Intersample Precision of ICP/MS

Sample	n	$MR_{87/85}$	SD
Urine, Natural	6	.3888	.0013
Urine, enriched	6	.8168	.0040
Red Cell, Natural	6	.3855	.0028
Red Cell, enriched	6	.8524	.0043
HCl + Rb natural	6	.4019	.0036
HCl + Rb enriched	5	.7786	.0027

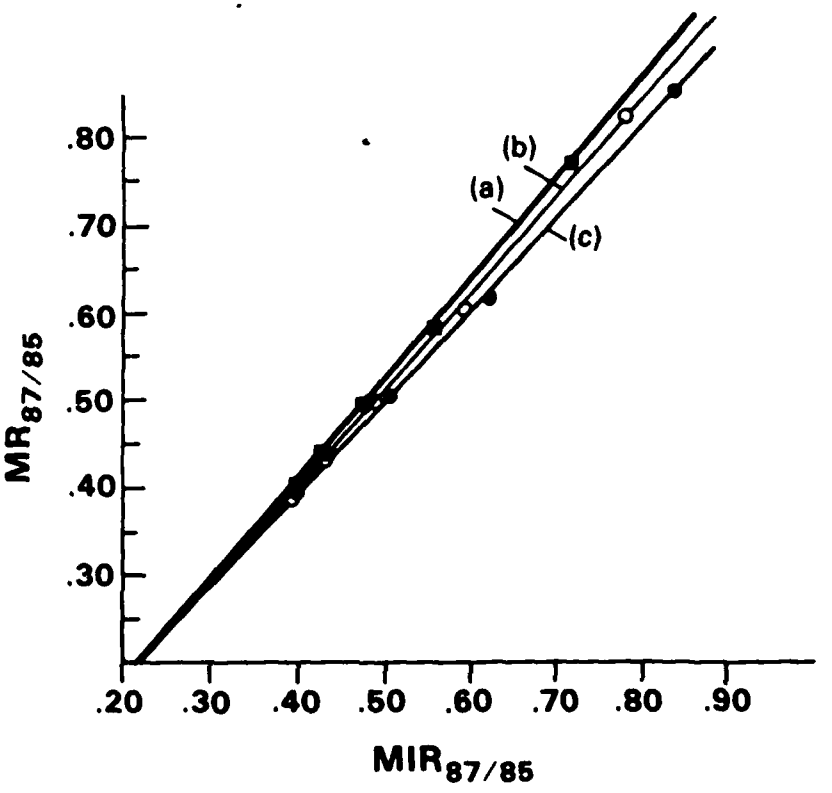

Fig. 4 Plots of $MR_{87/85}$ vs. $MIR_{87/85}$ for three matrices spiked with increasing amounts of enriched ^{87}Rb and processed as per Scheme of Fig. 1.

(a) 1N HCl matrix
(b) urine
(c) red cells

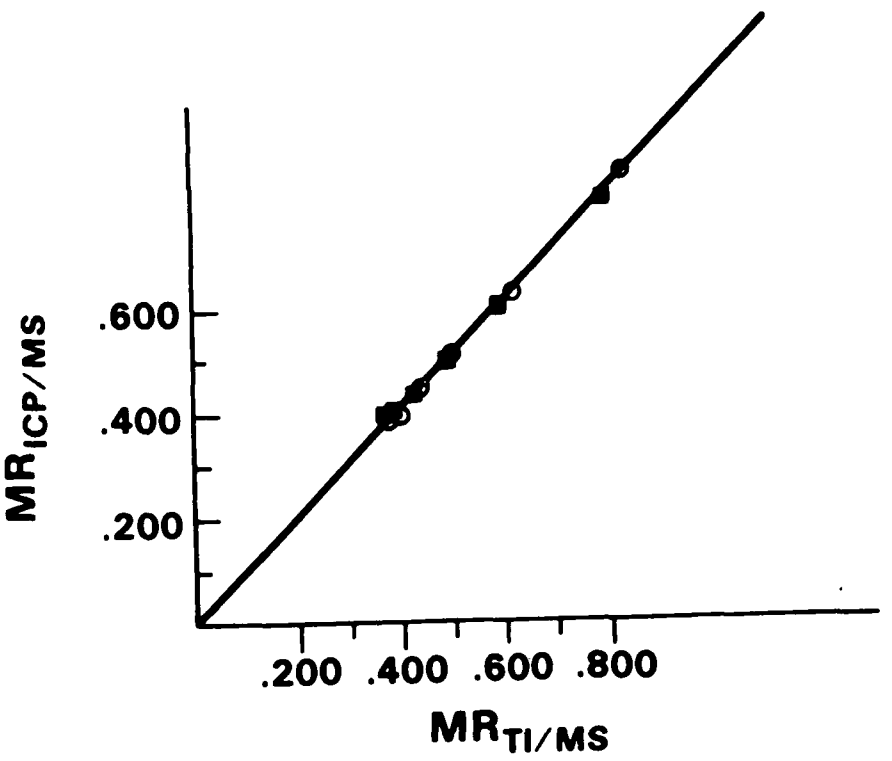

Fig. 5 - Comparisons between TI/MS and ICP/MS for Stable Isotopes of Rb

Table IV - Data Comparing Accuracy of Rb Analysis

Method	#Replications	ppm Rb ± 1SD
IDA	10	1.202 ± .038
Standard Curve	10	1.213 ± .015
Standard Addition	4	1.154 ± .068

Segment III - Stable Isotopes of Bromine

In relation to the present program, stable isotopes of bromine (^{79}Br, ^{81}Br) will serve two potentially important functions. First they will permit accurate measurement of the Extracellular Fluid Volume (ECFV) simultaneously with measurements of Total Body Water (TBW, method of $H_2{}^{18}O$), thereby making possible investigation of alterations in compartments of TBW in relation to studies of dehydration. And secondly, they will provide, for the first time, a tracer method to investigate dynamics of transport of chlorine, potential development of chlorine deficit, and Exchangeable Chlorine (CL_e), in relation to exercise in heat. At present, little is known about Cl_e in man, not because it is not important, but due to lack of suitable tracer methods.

We have now completed all the necessary analytical chemistry work for accurate measurement of the two stable isotopes of Br in fluids resulting from human studies. In this segment, we have briefly discussed these developments.

EXPERIMENTAL

Instrumentation. The ICP/MS employed in these studies was an Elan Model 250 system (SCIEX, Thornhill, Ontario, Canada). The nebulizer was of Meinhard concentric glass type, TR-30C (Meinhard Associates, CA). The distance from the load coil to the sampler was 27 mm (not adjustable). Sample solution was aspirated into the argon plasma via a peristaltic pump (Rabbit, Rainin Instrument Co., Inc., Woburn, MA) using approximately 150 cm length of tubing (Polyvinyl chloride, i.d. 0.76 mm, Rainin Instrument Co., Inc., Woburn, MA, USA). The total calculated volume of sample introduction tubing was 0.68 ml. Solution flow rate was approximately 1. ml/min.

Data acquisition was in the (peak hopping) isotope ratio mode. When operating in this mode, the instrument permits control of a number of parameters via the software. For the readers familiar with this instrument, the parameter set chosen was as follows: resolution (M), measurements/peak (3), scanning mode (I), measurements mode (M), measurement time sec (1.000), repeats/integration (10), dwell time ms (50), cycle time sec (0.400). For readers not familiar with this instrument, the above parameters result in the following operation. Upon initiation of the data acquisition cycle, the instrument will start at the center point less 0.05 amu for m/z=79. It will acquire data for 50 ms; will then hop to the center point and after 50 ms data acquisition proceed to the point 0.05 amu to the right of the peak position, collecting ions for an additional 50 ms. This sequence is then repeated for the peak at m/z=81, completing one cycle. The total time required for this is approximately 324 ms (4ms overhead time/point). Since cycle time was set at 0.400 s, the second data acquisition cycle will then begin after 400 ms has elapsed from the start of the first cycle. The number of cycles is repeated until the measurement time of 1.000 s/point is reached. Following completion of a cycle, data are processed and a printout is given containing information on ion intensities (ions/s) and the corresponding isotope ratios. This constitutes one integration and is repeated as many times as required (ten for the present parameters set). The average value of the ten sequential measurements of ion intensities/isotope ratios is taken as a single measurement and reported, where appropriate, together with ±1SD of the ten measurements. Unless noted otherwise, the Relative Standard Deviation (%RSD) is taken as an indication of the measurement precision.

Because of the significant background ion beam at m/z=81 ($^{40}Ar^{40}Ar^{1}H^{+}$), a Correction Factor (CF) is instituted via the software. The instrument is instructed to monitor the peak at m/s=76 ($^{40}Ar^{36}Ar^{+}$), in the same measurement sequence as for m/z=79,81. The CF, based on the ratio of ion beams at m/z=76,81 when deionized water is aspirated into the plasma, automatically subtracts the contribution of $^{40}Ar^{40}Ar^{1}H^{+}$ during each measurement cycle.

Prior to a day's run, our operational practice is to

optimize the instrument settings in the following order: lens settings, rf power, nebulizer pressure, plasma flow rate, and auxiliary flow rate. The settings vary for different days, but typical values used in this work were: rf power about 1000 watts; argon flow rates (nebulizer pressure in psi, auxiliary flow rate in L/min, and plasma flow rate in L/min, resp.) 42, 2.0, and 12.0. For these adjustments, we use a solution of 5.0 ug/ml Br as NaBr (natural) and monitor the ion intensity at m/z=79.

Isotope Calibration Procedures. The measured ratio of $^{81}Br/^{79}Br$ requires background correction due to $^{40}Ar^{40}Ar^{1}H^{+}$, and varies somewhat from the expected isotopic ratio depending on instrument parameters (see: RESULTS AND DISCUSSION). Since accurate measurement of the ratio is of fundamental importance to our applications, we have devised a calibration procedure for converting the corrected measured ratios in unknown samples (referred to as $MR_{81/79,C}$) to the expected true isotope ratios expressed on the mass scale (referred to as the Mass Isotope Ratio, $MIR_{81/79}$).

The calibration procedure consists of isotopic analyses of a set of isotope calibration standards prepared by incremental additions from a highly enriched ^{81}Br solution to solutions of natural Br such as to provide standard solutions whose Br concentration is in the range 4-5 ug/ml, but $MIR_{81/79}$ values vary over the expected range of the ratios for the unknown samples. A single stock solution of NaBr was prepared at the beginning of these studies and used throughout the work. similarly, a single stock solution of $Na^{81}Br$ was prepared and used throughout the experiments. Isotopic concentrations of these solutions were calculated based on the assumption of 100% chemical purity. All work reported in this manuscript referred to these two stock solutions as the reference standards.

Chemical Separation Schemes. We have developed two chemical separation schemes, Fig. 6a based on ion exchange separation for samples of plasma, red cells, and saliva and Fib. 6b based on distillation, for urine.

Others. Stable isotope of Br (^{81}Br, 97.81 at .%, as NaBr) was obtained from Oak Ridge National Laboratory (Oak Ridge, TN). All other chemicals were analytical reagent grade used as purchased.

RESULTS AND DISCUSSION

Background, Sensitivity, and Detection Limits. The present mode of operation of the mass spectrometer is in the positive ion mode. Sample introduction is via single-pass continuous nebulization of the analyte solution using a peristaltic pump at 1.0-1.5 ml/min. Two types of background are present in this region whose quantitative significance in relation to stable isotopes of Br needs resolution: true Br background of aqueous solutions and the ion background contribution due to $^{40}Ar^{40}Ar^{1}H^{+}$.

Fig. 6a

SCHEME I - for plasma, red cells, saliva

1. Place 5 ml sample in dialysis tubing (Spectro/Por #132680, MW Cutoff 12000-14000, Spectrum Medical Industries, Los Angeles, CA) tie both ends in knots to prevent leaking.

2. Place dialysis tubing inside polyethylene bag (Ziplock) containing 50 ml DI-water; close using dialysis closures.

3. Place bags on horizontal laboratory shaker; shake for 1.5-2.0 hours.

4. Decant outer solution into beaker; boil until volume is a few milliliters.

5. Apply to cation exchange column (Dowex 50-x8, 10-200 mesh, 15x75 mm, Bio. Rad Laboratories, Richmond, CA).

6. Elute with 25 ml DI-water; reduce volume if necessary by boiling.

7. Run on ICP/MS for $^{81}Br/^{79}Br$.

(Note: for saliva samples, proceed directly to step 5)

Fig. 6b

SCHEME II - Urine

1. Place 20 ml urine in distillation flask; add 10 ml Conc. HNO_3; fit with distillation column, condenser, and collection flask.

2. Heat on hot plate; collect initial 10-15 ml.

3. Run on ICP/MS for $^{81}Br/^{79}Br$.

We have examined these issues over the period of about a year. Our results have been summarized in Table V. The background contribution to m/z=79, when DI-water is run, is in the range 100-300 ions/s (Table V). This comprises all contributing ions including any arising from Ar, Kr, and natural contamination background of Br as well as overall noise background of the instrument. The ion intensity at this m/z for 1.0 ug/ml of natural Br is in the range 10,000-20,000 ions/s, leading to a calculated Detection Limit (DL, $3\sqrt{B}$) of 2-5 ng/ml (ppb). Total solution requirement for this mode of sample introduction is high (5-10 ml), so that the absolute DL of the method is 10-50 ng of natural Br. The Determination Limit ($10\sqrt{B}$), would be about three times the corresponding value for DL.

We have also examined the relative significance of Kr impurity from the argon gas supply by monitoring the ion beam intensities at m/z=81,84 (^{84}Kr, ab=57.0 at %) on a number of occasions. The intensity ratio for m/z=84 relative to m/z=81 has been 0.05 or less, leading us to conclude that the predominant background corrections are from argon peaks or Br present in the blank.

Ion collection rates for stable isotopes of Br are about 10-50 times less than we have observed for such trace elements as Fe, Zn, and Cu. Fortunately, in relation to the available Br concentrations in the fluids of interest, the method suffers from no limitation.

The ion intensities due to $^{40}Ar^{40}Ar^{1}H^{+}$(m/z=81) are in the range 600-7000 under the operating conditions used for routine measurements of stable isotopes of Br (Table V). Therefore appropriate corrections are required. One approach is the experimental determination of the correction factor for each sample via another argon peak. We have examined the ratio for the ions at m/z=76($^{40}Ar^{36}Ar^{+}$), and m/z=81 as a possible pair. The results show a significant day-to-day variation for this ratio (Table V). The reasons for this variation appear related to the effect of plasma operating conditions on the reaction between water and argon, and need further clarification.

Precision of Isotope Ratio Measurements. Measurement precision of the isotope ratios is one of the most important fundamental considerations in the applicability of stable isotope tracer methods. The requirements vary widely depending on the particular application and the nature of the metabolites of interest. For example, in the application of the stable isotope tracer method for the measurement of Total Body Water (TBW), with $H_2{}^{18}O$ as the tracer, the measurement precision for the ratio $^{18}O/^{16}O$ must be no worse than 0.01%. Typical data for Br have been given in Table VI. The data summarized in this table are for Br-solution containing 4.0 ug/ml of natural Br, or same concentration spiked additionally with ^{81}Br to correspond to $MIR_{81/79}$=0.9974). The measurements are given both for values corrected for the $^{40}Ar^{40}Ar^{1}H^{+}$($MR_{81/76}$ given for each measurement; designated as CF) or for uncorrected measurements; both data sets

Table V - Background, Sensitivity, and Detection Limit[1]

Date	I_{79}	I_{81} (Deionized Water)	$MR_{81/76}$	I_{79} (1.0 ug/ml Br)	Detection Limit (ug Br/ml)
3/17/86	245±12	4684±103	0.898	9732±70	0.0048
04/24	334±10	4486±94	0.433	18466±226	0.0030
06/02	230±7	7245±150	1.500	15564±235	0.0029
07/11	253±10	4338±45	0.806	13587±147	0.0035
08/13	171±8	3783±107	0.887	15340±115	0.0026
09/30	280±8	1191±88	0.332	11956±62	0.0042
10/31	70±4	602±8	0.326	11670±40	0.0022
11/12	133±9	900±19	0.391	17135±58	0.0020
12/12	109±6	851±16	0.333	12320±259	0.0025

[1]each data point corresponds to the mean ± 1SD of ten sequential measurements. Units for ion beam intensity: ions/s.

Table VI - Precision of Isotope Ratio Measurements

1-Uncorrected				
$MIR_{81/79}$=0.997	1.007±0.005	1.048±0.005		1.049±0.009
$MIR_{81/79}$=5.322	4.781±0.034	5.262±0.031	5.405±0.046	5.199±0.043
2-Corrected (CF*)	(0.333)	(0.403)	(1.500)	(0.433)
$MIR_{81/79}$=0.997	0.993±1.005	1.005±0.005		0.988±0.009
$MIR_{81/79}$=5.322	4.770±0.034	5.228±0.031	5.329±0.036	5.152±0.043

*Correction Factor (CF) as determined immediately prior to the run with deionized water as the sample.

referring to the same measurements. Several important points %RSD of the measurements was invariably better than 1%. There was no difference in this parameter between the natural or highly enriched solution. There was also no difference in this parameter between the corrected data and the corresponding uncorrected values.

We have examined the measurement precision for this ratio in biological fluids (plasma, urine, and saliva, Tables VIII, IX, XII, and XIII). The measurement precision is not different from the value obtained from simple solutions of bromide. Therefore, it is concluded that measurement precision of better than 1% (RSD) can generally be achieved readily and routinely with this method for the concentration range of interest to these applications.

The concentration range for natural Br over which constant values of $MR_{81/79,C}$ can be obtained is consistent with the available Br concentration in biological fluids (Table VII). At Br concentrations below about 1.0 ug/ml, the correction procedure does not appear to provide accurate data, but for the concentration range 3.0-20.0 ug/ml the values of $MR_{81/79,C}$ agreed to within the accepted measurement precision of the method, independently of Br concentration. Since the prevailing Br concentration in the biological materials of interest is above 1.0 ug/ml no need arises for preconcentration of Br.

Linearity. We have tested the linearity of isotope ratio measurements on several occasions during a year's time by use of a single set of stable isotope calibration solutions. These solutions contained natural Br concentration of 4.0 ug/ml to which had been added increments of ^{81}Br in order to achieve the isotope ratio range 0.9974-5.322. The linear regression parameters for the data have been summarized in Table VII both for isotope ratios corrected for the argon background at m/z=81 as well as uncorrected values.

All of the data clearly demonstrate a highly linear relationship between the two parameters. The intercept is close to zero, but somewhat higher for the uncorrected ratios as compared to when the argon background has been subtracted. The slope varies for different days of operation, sometimes deviating considerably from the expected value of unity. At this time, we have no clear explanation for this observation, but presume it to be related to changes in argon-plasma background resulting from daily differences in the operating conditions. Based on these data, it becomes clear that appropriate stable isotope calibration standards are a requirement for accurate measurement of stable isotope ratios with this technique.

Sulfate Interference. We have developed two schemes for the measurement of stable isotopes of bromine in biological fluids. The earlier scheme (Fig. 6a), was based on cation exchange for separation of Na from matrix constituents. The more recent procedure (Fig. 6b) relies on distillation of HBr from acidified

Table VII - Linear Regression Parameters for Stable Isotope Calibration Standards (Ion Exchange Separation)

Date	Slope (a)	Intercept (b)	r^2
1 - Corrected			
12/12/86	0.861	0.131	0.9997
09/09	0.962	0.017	0.9997
07/18	0.972	0.031	0.9997
07/02	1.003	-0.037	0.9995
04/24	0.958	0.028	0.9999
2 - Uncorrected			
12/12/86	0.861	0.144	0.9994
09/09	0.960	0.047	0.9997
07/18	0.976	0.064	0.9998
07/02	0.998	0.058	0.9996
04/24	0.956	0.087	0.9999

$MR_{81/79} = (a) \times (MIR_{81/79}) + b$

solutions of sample using HNO_3.

We had initially developed the ion exchange procedure, until we observed a consistent systematic difference in the values of $MR_{81/79,c}$ for urine compared with plasma (Table VIII). All the measurements given in Table VIII were carried out on the same day. the data are repeatable for different days. They are reproducible for any well-mixed pool of urine. Compared with the ratio for simple solutions of Br, values obtained for serum appear to be without systematic error, but for saliva and urine an increasing positive bias is observed. When samples of urine for different subjects collected on several occasions are compared (Table IX), considerable variability is observed. These changes in the measured ratio are not related to the effect of argon background correction as they are both highly reproducible and the urine bromine concentration (Table IX) is well above the 1.0 ug/ml cut-off point.

Sulfate concentration in human serum is reported at 30 ug/ml, but is of course both much higher and more variable in urine (several hundred to thousand ug/ml depending on the subjects' sulfur intake). Both subject-to-subject and day-to-day variations could also be large.

Data given in Table X show the quantitative aspects of the increase in ion intensity at m/z=81 due to increasing concentrations of sulfate. The assignments made as to the specific identity of the species present at m/z=50,81 are tentative and may not be inclusive. But as clearly demonstrated from the data given in Table X, sulfate could be responsible for the artifically high values observed for $MR_{81/79,c}$ in urine and to a lesser extent in saliva (Table VIII). Proceeding with the assumption that the error is largely due to sulfate, we attempted its removal with $Ba(NO_3)_2$ added in excess of stoichiometry to the urine prior to the ion exchange step of SCHEME I. The results of one such experiment have been summarized in Table XI.

The data demonstrate the ability of $Ba(NO_3)_2$ to remove a significant portion of sulfate from urine. However, it is also clear that the values of $MR_{81/79,c}$ after treatment with the precipitant are both considerably higher than interference-free values, and vary significantly for different subjects. Therefore, sulfate precipitation does not appear to provide sufficient removal of this interferent to permit satisfactory measurement of the isotope ratios in human urine.

The results from distillation experiments (Fig. 6b) have been summarized in Tables XII and XIII. The time course of bromine distillation from urine of one subject has been given in terms of both ion intensity for ^{79}Br and the value of $MR_{81/79,c}$ in Table XII. Corresponding data for distillation of a standard solution of Br have also been provided. The data summarized in Table XII for urine correspond to the mean±1SD for five independent replicatons (each consisting of 20 ml of urine) from a well-mixed urine pool. Those for the standard solution of Br

Table VIII - Comparison of $MR_{81/79,c}$ Values in one Subject for Different Fluids

Sample	I_{79}(i/s)	$MR_{81/79,c}$
1.0 ug/ml BR	13622	0.971±0.006
3.0	43684	0.982±0.005
Serum		
#1	16918	0.987±0.008
#2	17559	0.982±0.007
#3	18276	0.985±0.007
Parotid Saliva		
#1	22180	0.994±0.005
#2	19334	1.001±0.007
#3	21953	0.994±0.003
Urine		
#1	40611	1.066±0.009
#2	42218	1.073±0.006
#3	41982	1.074±0.007

Sample preparation according to SCHEME 1

Table IX - Inter- and Intra-individual Variabilities of Urine $MR_{81/79,c}$ Processed According to SCHEME I

Subject	I_{79}(i/s)	$MR_{81/79,c}$ for Day* 1	2	3	4	5
MJ	48000-89000	1.050± 0.009	1.010± 0.002	1.020± 0.001	1.038± 0.005	1.054± 0.003
DA	45000-74000	1.015± 0.003	1.021± 0.007	1.085± 0.004	1.027± 0.004	1.045± 0.008
BT	10200-43000	1.056± 0.003	1.044± 0.012	1.058± 0.003	1.069± 0.001	1.059± 0.003
1.0 ug/ml BR	14630	0.970±0.011				
4.0	66323	0.985±0.006				

*Data correspond to mean±1SD of independent measurements from three separate aliquots, each taken through the entire SCHEME 1

are for a single aliquot. It is clear from these data that highly reproducible measurements of $MR_{81/79,c}$ are obtained even in the fourth fraction of the distillate for urine samples. The values of $MR_{81/79,c}$ obtained from urine samples are identical with those measured for standard solution of Br. It is also of interest to note that the specific fraction collected from the distillate does not appear to pose an important issue.

Data presented in Table XIII summarize the reproducibility of these measurements, with the distillation procedure, for four different subjects. These data correspond to the mean±1SD for five replications of 10-ml aliquots. It is clear that highly reproducible measurements of $MR_{81/79,c}$ are obtained fro all subjects. The measured values are identical within the expected precision of the method for all four subjects, and compare very favorably with that obtained from replicate measurements of standard solution of Br.

Precision and Accuracy of Overall Analyses. In order to test the precision and accuracy of the complete procedure (Fig. 6b), 20-ml aliquots from a well-mixed pool of urine obtained from three individuals were spiked with known increments of natural bromine (as NaBr). Each spiked aliquot was then further spiked with known amount of highly enriched ^{81}Br (see METHODS). The details have been given in Table XIV.

The resultant samples were then treated as unknowns whose natural bromine content was then determined using the concepts of isotope dilution. The measured values of $MR_{81/79,c}$ were converted to the corresponding values of $MIR_{81/79}$ by comparison with the linear regression calibration plot obtained from stable isotope calibration standards. The following isotope dilution expression was used for the calculation of total Br in each (unknown) sample (true endogenous Br+ natural Br increment added).

$$X=[^{81}Br^{*}-(MIR_{81/79})\times(^{79}Br^{*})]/[0.5007(MIR_{81/79}-0.9974)]$$

X: ug Br in sample (20 ml urine)
$^{81}Br^{*}$, $^{79}Br^{*}$: ug isotope added to sample as in vitro spike
0.5007, 0.9974: constants related to natural isotopic composition of Br

The results from these analyses have been summarized in Table XV. Highly linear regression equations result for the relationship between the amount of Br found and the amount added as natural spike ($r^2>0.97$ for all subjects). Furthermore, when we subtract the amount of natural spike added to each urine sample (Table XIV) from the amount calculated from the isotope dilution procedure (Table XV), the corresponding mean±1SD for the five samples of each subject compare with the corresponding linear-regression intercept as follows (ug Br/ml of urine, first figure is based on subtraction of the spike): BT, 1.92±0.10 vs. 2.09; XFS, 3.82±0.09 vs. 3.93; and MJ, 8.56±0.19 vs. 8.38. The

Table X - Ion Intensities for Ion Peaks Relevant to the Sulfate Problem

Sulfate Concentration (ug/ml)	I_{50}	I_{76} (i/s)	I_{81}	I_{81} Due to Sulfate
0	87	3405	2320	0
1000	14602	2999	7421	5378
2000	28790	2863	13320	11369
3000	42410	2768	18845	16959
4000	71743	2515	28985	27271

Peak assignments: m/z=50, $^{34}S^{16}O^{+}$
76, $^{40}Ar^{36}Ar^{+}$
81, $^{40}Ar^{40}Ar^{1}H^{+}$, $^{32}S^{16}O_3{}^{1}H^{+}$

Table XI - Removal of Sulfate Interference from Human Urine Using $Ba(NO_3)_2$*

Subject	I_{79} (i/s)	I_{50}	$MR_{81/79,c}$ -Ba	$MR_{81/79,c}$ +Ba
MJ	48350	12285	1.113	
	43175	2284		1.012
XFS	14640	5372	1.178	
	14145	1094		1.068
BT	23370	14350	1.468	
	23275	2294		1.250
AS	41855	17830	1.131	
	40495	4315		1.052
SZ	30985	6113	1.059	
	30685	1105		1.010
1.0 ppm BR	13750	----	0.981	-----
4.0	60530	----	1.002	-----

*Measurement precision for all values of MR<<1%. All data are average measurements on two independent aliquots from a well-mixed pool of urine.

precision (±1RSD) of the five replications for each subject is in the range 2-5%. Thus, the overall precision of the complete analysis scheme for Br in human urine with the isotope dilution procedure is better than 5%. It is not possible to assign a figure of accuracy based on these data, but if the assumption is valid that complete isotopic exchange was achieved between the enriched *in vitro* spike and natural Br (the sum of added natural spike and endogenous Br), the overall accuracy must be comparable to the precision of the analyses. This is due to the expectation that the isotope ratios are measured with both precision and accuracies of about 1%.

If the isotope ratios can in fact be measured with precision and accuracies of about 1%, we should be able to expect the corresponding figures for quantitative isotopic analysis based on isotope dilution to be only somewhat larger. Therefore, we should expect overall accuracies considerably better than the upper limit of 5% observed for samples from BT. The precision for the other two subjects is about 2%.

CONCLUSIONS

This manuscript constitutes the first report of measurement of stable isotopes of Br in relation to human metabolic studies. It also is the first description of such studies with the new technique of ICP/MS. The results indicate that both stable isotopes of Br can be measured with precision and accuracy of 1% or better for the ratio, and 2-5% for absolute amounts.

The method should open up new possibilities for studies related to components of Total Body Water (TBW), exchangeable body chlorine for investigations of chlorine metabolism in man, metabolism of Br as a potentially interesting trace element in humans, and disturbances in the homeostasis of extracellular electrolytes in disease.

Table XII - Measurement of I_{79} and $MR_{81/79,c}$ in Aliquots of Urine after Distillation with HNO_3*

Collection Sequence	Urine		2.0 ppm BR Solution	
	I_{79}	$MR_{81/79}$	I_{79}	$MR_{81/79}$
1	29718±7755	1.001±0.003	110600	1.002
2	29392±4820	1.004±0.009	26020	1.008
3	27376±4324	1.004±0.017	11460	1.008
4	5515±3977	1.013±0.052	849	1.054
5	368± 128	0.981±0.023	212	1.043

*Procedure: 20 ml aliquot of urine (or Br standard) acidified with 10 ml conc. nitric acid and distilled. Fractions correspond to sequential collection of 5 ml. For urine, five such experiments were performed on aliquots taken from a well-mixed pool. Data are mean±1SD of the five replications.

Table XIII - Interindividual Reproducibility of $MR_{81/79,c}$ for Distillates from Human Urine

Subject	I_{79}	$MR_{81/79,c}$
XFS	37858± 4497	1.004±0.003
TZ	75378± 3361	1.003±0.002
MJ	125565±17600	1.005±0.0008
BT	33650± 6123	1.008±0.002
2.0 ppm BR	46474±15676	1.004±0.0008

*Mean±1SD for five independent replications from a single well-mixed pool for each subject. Procedure same as for Table 10.

Table XIV - Preparation of Urine Samples for Isotope Dilution Analysis[1]

Aliquot *	ug Natural Br Added	ug Enriched Br Added ^{81}Br*	^{79}Br*
0	0	0	0
1	0	244.6	5.35
2	7.99	"	"
3	15.97	"	"
4	23.96	"	"
5	31.94	"	"

[1]identical procedure was followed for urine from three subjects. (*) denotes that this material is isotopically enriched.

Table XV - Results of Isotope Dilution Analysis for Aliquots of Urine from Three Subjects

Sample * (Table XIV)	ug Br Calculated/20 ml for Subject BT	XFS	MJ
1	48.5[1]	78.6	164.5
2	47.8	85.1	179.8
3	55.3	92.7	189.1
4	60.9	99.3	198.1
5	67.6	106.0	204.3

[1]We assume that this sample received the unintended spike of 7.99 ug natural Br by mistake (same as for sample #2)

Segment IV - Stable Isotopes of Lithium

Lithium is of interest as a potentiating factor for inducing heat illness. Investigation of the mechanisms involved could permit advances in our understanding of the causes, and potential preventive measures, of this problem.

We have now completed the first phase of our studies with stable isotopes of lithium (^{6}Li, ^{7}Li). We have shown, for the first time, that ICP/MS will permit accurate measurement of these stable isotopes in biological fluids resulting from Li-administration protocols.

For the follow-up phase of this portion of the research, we are planning animal studies to investigate use of Li as a potentiating factor in heat illness.

EXPERIMENTAL

Instrumentation. The ICP/Ms employed in these studies was an Elan Model 250 system (SCIEX, Thornhill, Ontario, Canada), and operated as described in the previous segments.

Isotope Calibration Procedures. The measured ratio of $^6Li/^7Li$ varies somewhat from the expected isotopic ratio depending on instrument parameters (see: RESULTS AND DISCUSSION). Since accurate measurement of the ratio is of fundamental importance to our applications, we have devised a calibration procedure for converting the measured ratios in unknown samples (referred to as $MR_{6/7}$) to the expected true isotope ratios expressed on the mass scale (referred to as the Mass Isotope Ratio, $MIR_{6/7}$).

The calibration procedure consists of isotopic analyses of a set of isotope calibration standards prepared by incremental additions from a highly enriched ^6Li solution to solutions of natural Li such as to provide standard solutions whose Li concentration is in the range of 0.1-0.5 ug/ml, but $MIR_{6/7}$ values vary over the expected range of the ratios from the unknown samples. Isotope standards are analyzed in an intermittent fashion with the unknown samples. Additionally, as there were initial concerns with respect to the possibility of matrix effects, we have also prepared similar isotope calibration standards from natural matrices (human urine, plasma, red cells) spiked both with known amounts of natural Li and incremental additions of highly enriched ^6Li (see: RESULTS AND DISCUSSION).

Chemical Separation Scheme. Following initial evaluation of a number of separation procedures, we have adopted the procedure given in Fig. 7.

Stable Isotopes and Natural Lithium Standards. Highly-enriched $^6Li_2CO_3$ (wt% ^6Li=94.88) was obtained from Oak Ridge National Laboratory (Oak Ridge, TN, USA). Appropriate amount of the material was dissolved in deionized water, its resultant total Li-concentration measured with flame atomic absorption spectrophotometry (Perkin-Elmer Model 5000, Norwalk, CT, USA). The resultant stock solution was used in all experiments as the primary source of ^6Li. Similarly, natural-lithium standard solution (1000 ug/ml, Traceables, MCB Reagents, EM Science, NJ, USA) was employed as the stock solution for all natural lithium used in this work. All working standards were derived from these two sources of lithium. This assured internal consistency regardless of any possible systematic errors in the measurements of Li concentrations in the primary standards.

Others. All chemicals were analytical reagent grade used as purchased.

Fig. 7

SCHEME 1

1. For samples of red cells and serum (2-3 ml), digest using 10 ml conc. HNO_3, and similar quantity of H_2O_2. For urine samples (5 ml) skip steps 1-3 inclusively.

2. Evaporate to a small drop; add water and repeat 2-3 times) to remove as much acid as possible).

3. Adjust pH to 6.5-7.0 with KOH.

4. Apply solution to anion-cation double column*, add 20 ml water to the columns.

5. Disconnect anion-exchange column; elute Li from the cation-exchange column with about 40 ml 0.9 N HCl.

6. Evaporate solution until volume is about 25 ml; measure $^6Li/^7Li$ with ICP/MS.

7. Also measure isotope calibration standards intermittently with the unknowns.

8. Measure Li concentrations in the original sample with atomic absorption spectrophotometry.

*Column preparation:

Anion-exchange column: Dowex 1-X8 (Bio-Rad Laboratories, Richmond, CA, USA), 100-200 mesh, chloride form, 1X5 cm column filled to about 3 cm height.

Cation-exchange column: Dowex 50W-X16, 200-400 mesh, hydrogen form, 1x20 cm column filled to about 17 cm.

Poured columns are connected, then washed with 50 ml 6N HCl, followed by 50-100 ml water until pH is about 6.5.

RESULTS AND DISCUSSION

Development of an integrated analytical method for accurate isotopic analysis of Li with ICP/MS requires careful scrutiny of a number of issues. We have examined these issues below.

Instrumental Issues. Instrument parameters of significance to accurate measurement of isotope ratios are: various background and interference problems, achievable precision and stability of isotope ratio measurements, linear dynamic range of the instrument with respect to isotope ratios, and any matrix effects on the measured values of the isotope ratios.

Background and Interferences. Fortunately, background ions such as those generated from the argon-plasma, isobaric interferences, and interferences related to interactions between reagents and the argon-plasma appear not to play a major role for stable isotopes of Li. This is fortunate because the limited number of stable isotopes of this element does not permit selection of stable isotopes as is the case for other elements such as Se or Fe. Typical ion intensities observed for m/z=6,7 are listed in Table XVI for the cases of relevance to this application. Based on these data, the general background intensities recorded at m/z=6,7 correspond to 0.0003-0.0006 ug/ml of Li of natural composition. Lithium concentration of samples resulting from studies with manic-depressive patients is in the ug/ml range[3] so that the observed background intensities are negligible and do not necessitate corrections.

The effect of background ions on the measured ratio of the isotope pair of interest could be important for some trace elements. For lithium, this does not appear to constitute a limitation at natural Li concentrations $\geq$0.05 ug/ml (Table XVI). The data summarized in Table XVI have not been corrected for background. If this is done, it is clear that the resultant value of $MR_{6/7}$ will be significantly different from the uncorrected value only for Li concentration <0.05 ug/ml. For instance, at lithium concentrations of 0.01 or 0.10 ug/ml the background-corrected values of $MR_{6/7}$ are 0.0651 and 0.0631, respectively. These compare with the uncorrected values of 0.0667$\pm$0.0006 and 0.0632$\pm$0.0004, respectively. However, it is also clear from the data of Table XVI that $MR_{6/7}$ assumes a decreasing value with increasing lithium concentration. This is larger than the expected measurement precision for lithium concentrations $\leq$0.05 ug/ml. The reason for the observed change is not clear to us, but could be related to mass fractionation of the two isotopes. From a practical perspective, this is not a limitation as long as the lithium concentration is maintained above 0.10 ug/ml, and this can be readily accomplished.

Instrument Stability. An important issue related to potential utility of this technique for accurate isotopic analysis is the stability of the measured ion beam ratios ($MR_{6/7}$)

during the entire measurement period (typically up to eight hours). Although the present version of the instrument is capable of reasonable ion beam stability continuous operation over several hours entails significant ion beam drifts. As is evident from the data given in the table, ion intensities at m/z=7 underwent a continuous negative drift amounting to over 30% of the initial value during the 5-hour observation period. This coupled with unknown matrix effects preclude use of this method for accurate quantitative isotopic analysis at present time (unless in vitro isotope dilution analysis is employed). However, as clearly illustrated from the data of Table XVII, the measured ratios ($MR_{6/7}$) are independent of any instrument drift. The data given in the last column of the table indicate that no systematic drift took place and that the values of $MR_{6/7}$ were always within the expected measurement precision of the technique. This independence has also been shown for other trace elements where instrument ion beam drift is substantially greater than shown in Table XVII, and appears, at least at this time, to be a general characteristic of this method. Therefore, we conclude that the measured ion beam ratios ($MR_{6/7}$) are constant within the expected measurement precision of about 1% (RSD).

Dynamic Linear Range. The numerical value of the measured ion beam ratio for any given isotope pair (for Li: $MR_{6/7}$) is not necessarily identical with the corresponding true isotope ratio ($MIR_{6/7}$=0.0687). The measured ratio varies significantly for different instrument operating conditions. The magnitude of the deviation between these two parameters depends on the specific element. However, for accurate quantitative analysis this does not constitute a difficulty as long as a quantitative relationship between the two parameters ($MR_{6/7}$ vs. $MIR_{6/7}$) can be demonstrated. If this relationship were linear over the range of isotope ratios of interest to these studies, then the task of converting the values of $MR_{6/7}$ to their corresponding values of $MIR_{6/7}$ would, of course, have been simplified.

Data demonstrating the linear relationship between $MR_{6/7}$ and $MIR_{6/7}$ are given in Table XVIII. Four sets of data are given, each set obtained by spiking any given matrix of interest with a constant level of natural Li, but increasing levels of ^6Li in order to obtain a known but variable $MIR_{6/7}$. The resultant spiked samples were then processed according to Fig. 7. The data clearly demonstrates an important feature of the method: excellent dynamic linearity over the range of isotope ratio of interest to human metabolic studies. It should be noted here that the spiked standards had been processed according to the separation procedure (Fig. 7), so that lack of matrix effect is contingent upon removal of the major matrix constituents.

As will be shown later, the small differences observed in the linear regression parameters for different matrices (Tables XVIII, XIX) are not of quantitative consequence. This then indicates that it should be a relatively easy task to convert the measured values of $MR_{6/7}$ for any set of unknown samples to their corresponding values of $MIR_{6/7}$ through a simple calibration

Table XVI - Ion Intensity vs. Li Concentration and $MR_{6/7}$[1]

Li Conc. (ug/ml)	n	Time (min)	I_6 (ions/s)	I_7 (ions/s)	$MR_{6/7}$
0	10	0-30	42± 2	396± 5	0.106 ±0.004
0.01	5	30-45	743±31	11159±431	0.0667±0.0006
0.05	5	45-61	3254±56	50056±1093	0.0650±0.0009
0.10	5	61-74	7444±70	117752±651	0.0632±0.0004
0.50	3	74-86	37289±398	586150±5409	0.0636±0.0002
0	5	86-94	48± 2	479± 18	0.0996±0.003

[1]Each set of data corresponds to mean of multiplicate (n=3-10) serial measurements, each consisting of ten sequential measurements (see Experimental Section).

Table XVII - Ion Beam Stability and its Effect on $MR_{6/7}$[1]

Time (min.)	Ion Intensity for m/z=7 (ions)	%Δ	$MR_{6/7}$	%Δ
0-3	175280±7440	0.0	0.0650±0.0006	0.0
27-30	190415±4216	+8.6	0.0653±0.0011	+0.5
53-56	180266±8422	+2.8	0.0649±0.0006	-0.2
78-81	158481±6467	-9.6	0.0652±0.0004	+0.2
106-109	165770±2613	-5.4	0.0650±0.0006	0.0
135-138	154366±2118	-11.9	0.0657±0.0006	+1.1
166-169	141809±2413	-19.1	0.0657±0.0011	+1.1
193-196	133608±3331	-23.8	0.0653±0.0008	+0.5
218-221	116938±1127	-33.3	0.0654±0.0007	+0.6
250-253	121972±3450	-30.4	0.0649±0.0007	-0.2
300-303	120821±3144	-31.1	0.0644±0.0006	-0.9

[1]data are for a 1 ug/ml Li solution run continuously for 5 hours. Each data point corresponds to the mean±SD of ten sequential measurements.

procedure. For isotope calibration standards, water serves as a proper matrix. This simplifies preparation of calibration standards considerably.

Overall Matrix Effects on $MR_{6/7}$. Biological matrices of interest to these studies are complex, presenting many as yet unknown interactions with the argon-plasma. These interactions could modify the measured ratios of any given isotope pair to the extent of introducing significant errors of measurement. We have investigated the overall effect of matrix on the measured ratio ($MR_{6/7}$) at different isotope ratios for various matrices of interest to this project. The results of these investigations have been summarized in Table XIX. These data were obtained by spiking subsamples of the matrices of interest with Li (natural) and ^6Li in such a manner as to achieve the desired isotope ratio. The so-spiked samples were then processed according to Fig. 7. It is clear from these data that there are no major intermatrix effects for $MR_{6/7}$. As has also been discussed above in relation to isotope standards, the linear regression parameters of the isotope calibration plots appear to be, in large part, independent of the matrix. In addition, as will be discussed in a subsequent section use of any of the matrices for the preparation of isotope calibration standards yields identical results with respect to isotopic content of unknown samples. These observations taken collectively support the suggestion that matrix effect is not an issue of concern to quantitatively accurate isotopic ratio analysis for Li as long as the proposed separation scheme (Fig. 7) is employed.

Accuracy of Isotopic Analyses. The overall accuracy of the isotopic procedure was tested on subsamples of urine, red cells, and serum each of which had been spiked with a known amount (25.9 ug) of natural lithium. Each spiked subsamples was treated according to Fig. 7 with ^6Li-spiked standards were prepared from biological matrices as well as deionized water. The elemental content of each test subsample was then determined using each series of isotope-spiked calibration plots separately. The results of these analyses have been summarized in Table XX. The data demonstrate that the overall reproducibility of the analytical procedure is 1-2%. Furthermore, absolute determinations are accurate to better than 3%. There are no systematic errors resulting from matrix effects, so that preparation of matrix-matched spiked-standards does not appear to be a necessity. This is consistent with similar studies carried out with this technique for other trace elements. It should be emphasized that the data given in Table XX are based on the concept of isotope dilution analysis and thus any incomplete recoveries inherent in the scheme are not reflected in the results nor are they cause for concern.

Table XVIII - Linear Regression Parameters for the Relationship Between $MR_{6/7}$ and $MIR_{6/7}$

Spiked Matrix	a	b	r^2
Water	1.362	-0.0318	0.998
RBC	1.332	-0.0248	0.9991
Urine	1.336	-0.0307	0.9998
Serum	1.386	-0.0369	0.9984

[1] $Mr_{6/7}=(a)x(MIR_{6/7})-b$

$MIR_{6/7}$ is the value of isotope ratio (wt/wt) as determined from a knowledge of the total amounts of ^6Li and ^7Li introduced in each solution.

Table XIX - Matrix Independence of $MR_{6/7}$

$MIR_{6/7}$*	Sample Matrix (Water)	(Serum)	(Urine)	(RBC)
0.0687	0.0610 ±.0008	0.0616 ±.0006	0.0598 ±.0008	0.0608 ±.0007
0.1439	0.1570 ±.0016	0.1544 ±.0020	0.1553 ±.0015	0.1558 ±.0025
0.2946	0.3517 ±.0052	0.3506 ±.0036	0.3422 ±.0042	0.3514 ±.0032
0.4526	0.5383 ±.0037	0.5308 ±.0050	0.5370 ±.0070	0.5349 ±.0080
0.5913	0.7225 ±.0040	0.7181 ±.0078	0.7005 ±.0064	0.7135 ±.0076
0.7954	0.9933 ±.016	0.9963 ±.013	0.9696 ±.0094	0.9693 ±.014

*$MIR_{6/7}$: Calculated value of isotope ratios (wt/wt) based on the measured amounts of isotopes used in preparing each solution.

CONCLUSIONS

Accurate measurement of the two stable isotopes of Li is a prerequisite in the studies of Li-pool sizes. This requirement can now be met with a method which is also capable of a relatively high sample throughput. This is in clear contrast to such other methods of isotope ratio mass spectrometry as Thermal Ionization Mass Spectrometry (TI/MS) which suffer from the major limitation of unrealistically low sample throughput to permit conduct of human studies. This new method of *in vivo* Stable Isotope Dilution (*in vivo* SID) should permit investigation of whether blood lithium concentrations represent an accurate reflection of body lithium stores.

Table XX - Recoveries of Li Added to Subsamples of Various Matrices and Analyzed by Isotope Dilution Analysis[1]

Sample Matrix	n	Matrix of ^{6}Li-Spiked Standard			
		D.I. Water	Urine	Serum	RBC
Urine	6	25.2±0.3	24.7±0.3	25.0±0.3	25.5±0
RBC	6	25.2±0.3	24.5±0.3	24.7±0.3	25.2±0
Serum	6	24.6±0.5	24.1±0.5	24.3±0.5	24.8±0

Manuscripts Resulting From This Work

1. Janghorbani, M, TA Davis, and BTG Ting. Measurement of Stable Isotopes of Bromine in Biological Fluids with Inductively Coupled Plasma Mass Spectrometry, Analyst, 1987, In Press.

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